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Rapid Catalase Supplemental Test for Identification of Members of the Family *Enterobacteriaceae*

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A simple, rapid, semiquantitative slide catalase test useful for differentiating members of the family *Enterobacteriaceae* is described. Judging by the time required for appearance of oxygen bubbles in 3% hydrogen peroxide, the immediate catalase reactors were *Yersinia*, *Serratia*, *Proteus*, *Morganella*, *Providencia*, *Cedecea*, and *Hafnia* spp. The delayed catalase reactors were *Escherichia*, *Shigella*, *Klebsiella*, *Enterobacter*, *Salmonella*, *Citrobacter*, *Edwardsiella*, *Kluyvera*, and *Tatumella* spp. This information is especially useful for differentiating *Serratia* from *Enterobacter* spp. and *Yersinia* from *Escherichia* and *Shigella* spp.

Miniaturized and automated systems designed to identify members of the family *Enterobacteriaceae* have become commonplace. While these systems are capable of providing identifications within a few hours, the first identification generated may not meet the minimum confidence level recommended by the manufacturer of the system for a final identification. In such situations, it becomes necessary to perform one or more supplemental tests to arrive at the final identification. Most of the supplemental tests used require overnight incubation, which negates the rapid identification potential of a system. Many laboratories no longer even carry these supplemental tests, e.g., Jordan tartrate, sodium mucate, potassium thiocyanate utilization, and xylose fermentation tests. However, by determining the catalase activity of an isolate, the best identification from the choices listed by a system can be made in a few seconds and with readily available materials. Rapid catalase testing has previously been shown to be a practical aid, not only for the identification of the *Enterobacteriaceae* (3), but also for the identification of mycobacteria (2) and oxidative, nonsaccharolytic gram-negative bacteria (1, 4). Since 1972, when the first report on catalase activity of the *Enterobacteriaceae* was published (3), many new genera and species of this family have been described. This report presents data on the catalase activity of many of these additional members so that users of identification systems for the *Enterobacteriaceae* can incorporate catalase activity as a rapid supplemental test.

Table 1 lists the species and number of isolates of each member of the *Enterobacteriaceae* studied and shows the number of isolates falling into each of the semiquantitative categories of catalase activity. Whenever possible, fresh clinical isolates were tested. However, due to their infrequent occurrence, many or all isolates of some of the species tested were stock cultures (Table 1).

Colonies from 18- to 24-h 5% sheep blood agar and MacConkey agar plates (BBL Microbiology Systems, Cockeysville, Md.) were used for the rapid catalase test. The methodology is basically the same as previously described (1).

By using a bacteriological loop (cotton swabs and wooden applicator sticks obscure the reaction), a single colony was placed in a drop of 3% hydrogen peroxide on a glass slide (several colonies when each is less than 1.0 mm in diameter, viz., *Yersinia pseudotuberculosis* and *Tatumella ptyseos*), and the time required for the appearance of bubbles was noted. The instant appearance of bubbles was called immediate. Any delay of bubbling, no matter how slight, was called delayed. In most cases of delayed bubbling, at least a full second or more passed before a positive reaction occurred. As stated previously (1), the small and delayed bubbling from peroxidase in erythrocytes and leukocytes of sheep blood agar does not interfere with semiquantitative catalase interpretation.

For most of the isolates examined, each author separately tested two colonies growing on 5% sheep blood agar and two colonies growing on MacConkey agar, resulting in eight tests per isolate.

To ensure quality control of the 3% H_2O_2, daily testing was done with *Escherichia coli* ATCC 25922 for the delayed reaction, *Proteus vulgaris* ATCC 13315 for the immediate reaction, and *Streptococcus pyogenes* ATCC 19615 for a negative reaction.

Most or all isolates of *Cedecea* spp., *Enterobacter gergoviae*, *E. sakazakii*, *Citrobacter amalonaticus*, *Escherichia vulneris*; *Klebsiella ozaenae*, *K. rhinoscleromatis*, *Serratia odorifera*; and *Tatumella* spp. and *Kluyvera* spp. were kindly submitted by D. J. Brenner (Center for Infectious Diseases, Centers for Disease Control, Atlanta, Ga.). Some of the *Yersinia enterocolitica* isolates, as well as those of *Y. kristensenii* and *Y. frederiksenii*, were from the collection of S. Winblad (Malmö, Sweden), while the isolates of *Y. intermedia* were kindly submitted by E. J. Bottone (The Mount Sinai Hospital, New York). The isolates of *Y. pseudotuberculosis* were from the collection of Analytab Products, Plainview, N.Y. The *Y. pestis* isolate was ATCC 19428.

Using the time of appearance of a positive reaction, we distinguished two major groups of genera (Table 1). One group was the immediate catalase group, composed of *Serratia*, *Cedecea*, *Yersinia*, *Proteus*, *Morganella*, *Providencia*, and *Hafnia* spp. The other group was the delayed catalase group, containing *Escherichia*, *Shigella*, *Edwardsiella*, *Salmonella*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Kluyvera*, and *Tatumella* spp. Our results with *Escherichia*, *Shigella*, *Edwardsiella*, *Salmonella*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Proteus*, and *Providencia* spp. were identical to those obtained by Taylor and Achanzar (3).

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TABLE 1. Semiquantitative catalase activity of the *Enterobacteriaceae*

Isolate	No. of isolates tested		No. of isolates giving catalase reaction of:	
	Initial isolation	Stock culture	Immediate	Delayed
Cedecea davisae[a]	2	2	4	0
C. lapagei[a]	0	2	2	0
Edwardsiella tarda	4	1	0	5
Citrobacter amalonaticus[a]	5	0	0	15
C. diversus[a]	14	1	0	15
C. freundii	14	1	0	15
Enterobacter aerogenes	12	0	0	12
E. agglomerans	9	2	0	11
E. cloacae	26	0	0	26
E. gergoviae[a]	3	10	0	13
E. sakazakii[a]	4	9	0	13
Escherichia coli	120	0	0	120
E. vulneris[a]	0	2	0	2
E. hermannii[a]	1	0	0	1
E. fergusonii[a]	1	0	0	1
Hafnia alvei[a]	5	1	6	0
Klebsiella oxytoca	6	0	0	6
K. ozaenae	3	3	0	6
K. pneumoniae	52	0	0	52
K. rhinoscleromatis	1	4	0	5
Kluyvera ascorbata[a]	1	2	0	3
K. cryocrescene[a]	0	2	0	2
Morganella morganii	15	0	15	0
Proteus mirabilis	37	0	37	0
P. penneri[a]	2	2	4	0
P. vulgaris	8	0	8	0
Providencia alcalifaciens	8	0	8	0
P. rettgeri	12	0	12	0
P. stuartii	17	0	17	0
Salmonella arizonae	2	1	0	3
S. cholerae-suis	2	2	0	4
S. enteritidis	12	3	0	15
S. typhi	0	4	0	4
Shigella boydii	0	3	0	3
S. dysenteriae	0	4	0	4
S. flexneri	0	5	0	5
S. sonnei	5	6	0	11
Serratia liquefaciens	10	0	10	0
S. marcescens	24	0	24	0
S. odorifera[a]	2	4	6	0
S. rubidaea[a]	2	0	2	0
Tatumella ptyseos[a]	0	2	0	2
Yersinia enterocolitica[a]	6	15	21	0
Y. frederiksenii	0	1	1	0
Y. intermedia[a]	13	1	14	0
Y. kristensenii[a]	0	2	2	0
Y. pestis[a]	0	1	1	0
Y. pseudotuberculosis[a]	0	9	9	0

[a] Catalase activity not previously reported.

Users of miniaturized and automated identification systems for the *Enterobacteriaceae* will find the rapid catalase test valuable, rapid, and simple. It must be stressed that such determinations should be restricted to isolates grown on 5% sheep blood agar or MacConkey agar, since our preliminary studies showed that some isolates of species in the immediate category gave delayed reactions and some isolates of delayed species gave immediate reactions when grown on Mueller-Hinton agar.

Table 2 illustrates the utility of the rapid catalase test as a supplement for the commonly used API 20E miniaturized identification system (Analytab Products). The situations shown in this table demonstrate the ability of the rapid

TABLE 2. Use of the rapid catalase test with the API 20E miniaturized identification system to differentiate members of the *Enterobacteriaceae*

API profile confirmation no. and species tested[a]	Frequency	Type of catalase reaction	Additional tests (% accuracy)[b]
1305563			
E. cloacae	1/143	Delayed	DNA (0%), malonate (81%), sodium mucate (76%)
S. liquefaciens	1/202	Immediate	DNA (86%), malonate (3%), sodium mucate (0%)
1144522			
Y. intermedia	1/502	Immediate	Sodium mucate (0%), motility (0%)
E. coli	1/728	Delayed	Sodium mucate (80%), motility (75%)
1004110, serology			
Y. pseudotuberculosis	1/84	Immediate	Xylose fermentation (99%), Jordan tartrate (30%)
S. sonnei	1/334	Delayed	Xylose fermentation (0%), Jordan tartrate (99%)

[a] Good likelihood but low selectivity. The source of API profile numbers was *Analytical Profile Index: Enterobacteriaceae and Other Gram-Negative Bacteria*, 1985.

[b] Require several to 48 h.

catalase test to differentiate members of the genus *Serratia* from those of *Enterobacter* and to distinguish members of the genus *Yersinia* from those of *Escherichia* and *Shigella*. It can be seen that the catalase test separates these genera in a moment, as opposed to the hours or even days of incubation required by supplemental tests recommended by the manufacturer. In addition, the catalase test provides a clearer separation of the genera in question than do the recommended supplemental tests, as evidenced by the percentages in Table 2. Finally, the catalase test is inexpensive to perform in comparison with such supplemental tests as the Jordan tartrate and sodium mucate tests, whose substrates are not generally available in microbiology laboratories.

It must also be stressed that while our data clearly show the rapid catalase test to be extremely useful in differentiating *Escherichia* spp. from *Yersinia* and *Shigella* spp. and *Serratia* from *Enterobacter* spp., results of this test with those taxa represented in this study by only a few isolates should be considered an additional piece of information and not a final determinant. When the present database can be enlarged, it may be possible to expand the use of the rapid catalase test as a final differential test for distinguishing other genera of the *Enterobacteriaceae*, e.g., *Enterobacter* from *Cedecea* spp. or *Hafnia* from *Kluyvera* spp.

It would be useful to study the catalase activity of the *Enterobacteriaceae* on various selective media. If the same catalase reactions occur with isolates grown on selective media as with isolates grown on blood agar and MacConkey agar, catalase testing could be used to quickly and cheaply distinguish specifically sought pathogens from look-alike species. For example, on xylose-lysine-deoxycholate agar and Hektoen enteric agar, shigellae (delayed catalase reaction) could easily be distinguished from similar colonies of immediate reactors such as *Pseudomonas*, *Proteus*, *Providencia*, and *Serratia* spp. This distinction was mentioned in a previous study, although it was not clear whether the experiment was actually done or simply theorized (3).

We have briefly presented two possible uses for rapid catalase testing. It remains for the ingenuity of the clinical microbiologist to find additional uses for this simply and quickly interpreted test.

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